NBDPS Methylation Study External Quality Assessment Protocol
Approved May 2012

Create a standard protocol using control samples for external quality assessment (EQA). The primary objective of establishing EQA is to ensure that each lab actively involved in testing NBDPS samples is proficient in their respective laboratory techniques independent of the source material or extraction procedure.

The protocol differs depending on the type of methylation study being completed.

Genome-Wide Arrays:

- Bisulfite conversion will typically be completed using kits from either
  - Zymo Research - EZ DNA Methylation (specified in Illumina protocol)
  - QIAGEN - EpiTect (allows longer storage)

  Other commercial kits can be used but beware of home brew kits (lower QC)
- Batch entire process – convert all samples at the same time using the same kit with reagents from the same lot, when possible
- Each array can accommodate 12 samples; require 1 CEPH sample per array for EQA using the same sample for each array and each lab. If a Core Facility is used, require reporting of results from 1 technical control per array from a minimum of ~~3~~2 arrays for EQA. To build a comparison dataset of results for the common CEPH sample, it is requested (but not required) that each lab include the common CEPH sample on each array for all arrays run during their actual study and report these results.
- No negative controls are included on these arrays
- Laboratories should report results from all methylation sites and samples tested. The protocol will be used prior to initiating methylation studies using NBDPS repository samples.
- The same methods/platforms that will be used with NBDPS samples will be required.
- The following QC should be performed on Illumina data (these steps are strongly encouraged but are not required for EQA):
  - Removal of samples with low signal (sample has total signal intensity that is <50% of the median signal for all samples)
  - Removal of data points with detection p-values >.001
    This can be accomplished in R via the cpg.qc function in CpGassoc (available at http://genetics.emory.edu/conneely)

- CEPH Samples Run on Each Array Will Allow:
  - Intra-lab comparison of results from duplicate samples
  - Inter-lab comparison of methylation sites that labs assay in common, when possible

- Standards Required to Pass EQA:
  - 95% bisulfite conversion rates
  - 95% CpG site call rate
  - 99% concordance between CEPH (or other technical control) sample results for all CpG sites across the genome for intra-lab comparison of results, where

concordance is computed as the percentage of sites with beta values differing by <0.1

- 95% concordance between CEPH (or other technical control) sample results for all CpG sites across the genome for inter-lab comparison of results, where concordance is computed as the percentage of sites with beta values differing by <0.2

Candidate Gene Studies (e.g., Pyrosequencing, EpiTYPER, Bisulfite Sequencing)

- Bisulfite conversion will typically be completed using kits from either
  - Zymo Research - EZ DNA Methylation (specified in Illumina protocol
  - QIAGEN – EpiTect (allows longer storage)

  Other commercial kits can be used but beware of home brew kits (lower QC)
- Batch entire process – convert all samples at the same time using the same kit with reagents from the same lot, when possible
- Run using 96 or 384-well plates; require 1 CEPH sample per array for EQA using the same sample for each array and each lab; require enzymatically-methylated samples from QIAGEN at 0%, 50%, and 100% run in duplicate or triplicate for a standard curve. If a Core Facility is used, require reporting of results from 1 technical control per array from a minimum of ~~3~~2 arrays for EQA. To build a comparison dataset of results for the common CEPH sample, it is requested (but not required) that each lab include the common CEPH sample on each array for all arrays run during their actual study and report these results.
- Negative (no template) controls are included on each plate
- Laboratories should report results from all methylation sites and samples tested. The protocol will be used prior to initiating methylation studies using NBDPS repository samples.
- The same methods/platforms that will be used with NBDPS samples will be required.

- CEPH Samples Run on Each Plate Will Allow:
  - Intra-lab comparison of results from duplicate samples
  - Inter-lab comparison of methylation sites that labs assay in common, when possible

- Standards Required to Pass EQA:
  - Assess bisulfite conversion rate by a platform-specific method
  - > 50% CpG site call rate (lower call rate but successful calls are reliable)
  - 90% concordance between CEPH (or other technical control) sample results for all CpG sites investigated, where concordance is computed as the percentage of sites with beta values differing by <0.1 for intra-lab comparison of results and differ by <0.2 for inter-lab comparison of results.
  - Samples used to generate the standard curve should be within 10% of the target methylation amount (0%, 50%, and 100%)
  - No results reported for negative (no template) controls

CEPH sample ID = NA12335
http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ind.cgi?ind_id=94

If a laboratory does not pass EQA standards, they must discontinue all methylation assays and repeat EQA. If the lab does not pass EQA standards a second time, no manuscripts will be completed until the problems are identified and resolved.

Results reported to CDC are final (e.g., if errors are made transcribing data to results template and the data do not meet the standards required to pass EQA, the lab must repeat EQA).